

What is claimed is:

1. A non-oligomerizing tandem fluorescent protein, comprising a first monomer of a fluorescent protein operatively linked to at least a second monomer of the fluorescent protein, wherein the propensity of the tandem fluorescent protein to oligomerize is reduced or inhibited as compared to a monomer of the fluorescent protein.
2. The non-oligomerizing tandem fluorescent protein of claim 1, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.
3. The non-oligomerizing tandem fluorescent protein of claim 2, wherein the fluorescent protein is a *Discosoma* RFP or a fluorescent protein related to a *Discosoma* RFP.
4. The non-oligomerizing tandem fluorescent protein of claim 3, wherein the *Discosoma* RFP is DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12 or a mutant of SEQ ID NO:12.
5. The non-oligomerizing tandem fluorescent protein of claim 3, wherein the *Discosoma* RFP is a mutant of DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12, further comprising an I125R mutation.
6. The non-oligomerizing tandem fluorescent protein of claim 2, wherein the fluorescent protein is an *Aequorea* GFP, a *Renilla* GFP, a *Phialidium* GFP, or a fluorescent protein related to an *Aequorea* GFP, a *Renilla* GFP, and a *Phialidium* GFP.
7. The non-oligomerizing tandem fluorescent protein of claim 6, wherein the fluorescent protein related to the *Aequorea* GFP is a cyan fluorescent protein (CFP), or a yellow fluorescent protein (YFP), or a spectral variant of the CFP or the YFP.

8. The non-oligomerizing tandem fluorescent protein of claim 6, wherein the fluorescent protein related to the *Aequorea* GFP is an enhanced GFP (EGFP; SEQ ID NO: 4), an enhanced CFP (ECFP; SEQ ID NO: 6), an EYFP-V68L/Q69K (SEQ ID NO: 10), or an enhanced YFP (EYFP; SEQ ID NO: 8).

9. The non-oligomerizing tandem fluorescent protein of claim 1, wherein the fluorescent protein further comprises a mutation of an amino acid residue corresponding to A206, L221, F223, or a combination thereof of SEQ ID NO: 2.

10. The non-oligomerizing tandem fluorescent protein of claim 10, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 2.

11. The non-oligomerizing tandem fluorescent protein of claim 10, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 6 or SEQ ID NO: 10.

12. The non-oligomerizing tandem fluorescent protein of claim 1, wherein the first monomer and the second monomer are operatively linked using a peptide linker.

13. The non-oligomerizing tandem fluorescent protein of claim 12, wherein the fluorescent protein is DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO:12.

14. The non-oligomerizing tandem fluorescent protein of claim 13, wherein the peptide linker has an amino acid sequence as set forth in SEQ ID NO:26.

15. The non-oligomerizing tandem fluorescent protein of claim 1, further comprising at least a third monomer of the fluorescent protein, which is operatively linked to the first monomer or the second monomer.

16. A fusion protein, comprising the non-oligomerizing tandem fluorescent protein of claim 1 operatively linked to at least one polypeptide of interest.
17. The fusion protein of claim 16, wherein the non-oligomerizing tandem fluorescent protein is linked to the polypeptide of interest through a peptide bond.
18. The fusion protein of claim 17, wherein the non-oligomerizing tandem fluorescent protein is linked to the polypeptide of interest through a linker molecule.
19. The fusion protein of claim 16, wherein the at least one polypeptide of interest comprises a peptide tag.
20. The fusion protein of claim 19, wherein the peptide tag is a polyhistidine peptide.
21. The fusion protein of claim 16, wherein the polypeptide of interest is a cellular polypeptide.
22. The fusion protein of claim 16, wherein the polypeptide of interest is an enzyme, a G-protein, a growth factor receptor, or a transcription factor.
23. The fusion protein of claim 16, wherein the polypeptide of interest is one of two or more proteins that associate to form a complex.
24. A kit, comprising at least one non-oligomerizing tandem fluorescent protein of claim 1.
25. The kit of claim 24, comprising a plurality of different non-oligomerizing tandem fluorescent proteins.
26. The kit of claim 24, wherein the non-oligomerizing tandem fluorescent protein comprises a fusion protein.

27. A polynucleotide encoding the non-oligomerizing tandem fluorescent protein of claim 1.

28. A polynucleotide encoding the non-oligomerizing tandem fluorescent protein of claim 4.

29. A vector, comprising the polynucleotide of claim 27.

30. A host cell containing the polynucleotide of claim 27.

31. A kit, comprising at least one polynucleotide of claim 27.

32. A recombinant nucleic acid molecule, comprising the polynucleotide of claim 27 operatively linked to at least a second polynucleotide.

33. The recombinant nucleic acid molecule of claim 32, wherein the at least second polynucleotide comprises a transcription or translation regulatory element.

34. The recombinant nucleic acid molecule of claim 32, wherein the at least second polynucleotide encodes a polypeptide of interest.

35. A vector, comprising the recombinant nucleic acid molecule of claim 32.

36. The vector of claim 35, wherein the vector is an expression vector.

37. A host cell containing the recombinant nucleic acid molecule of claim 32.

38. A kit, comprising at least one recombinant nucleic acid molecule of claim 32.

39. The kit of claim 38, wherein the at least second polynucleotide comprises a restriction endonuclease recognition site or a recombinase recognition site.

40. The kit of claim 38, wherein the at least second polynucleotide encodes a polypeptide of interest.

41. The kit of claim 40, wherein the at least second polynucleotide encodes a peptide tag.

42. The kit of claim 38, comprising a plurality of different recombinant nucleic acid molecules.

43. A tandem non-oligomerizing fluorescent protein, comprising:  
     a donor, comprising a first fluorescent protein,  
     an acceptor, comprising a second fluorescent protein, and  
     a peptide linker moiety operatively linking the donor and the acceptor,  
 wherein the first fluorescent protein and second fluorescent protein are different,  
 wherein at least the first fluorescent protein or the second fluorescent protein is a non-oligomerizing tandem fluorescent protein of claim 1,  
 wherein cyclized amino acids of the donor emit light characteristic of the donor, and  
 wherein the donor and the acceptor exhibit fluorescence resonance energy transfer when the donor is excited, and the linker moiety does not substantially emit light to excite the acceptor.

44. The tandem non-oligomerizing fluorescent protein of claim 43, wherein each of the first fluorescent protein and the second fluorescent protein is a non-oligomerizing tandem fluorescent protein.

45. The tandem non-oligomerizing fluorescent protein of claim 43, wherein the non-oligomerizing tandem fluorescent protein comprises a *Discosoma* RFP or a fluorescent protein related to a *Discosoma* RFP.

46. The tandem non-oligomerizing fluorescent protein of claim 45, wherein the *Discosoma* RFP is DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12 or a mutant of SEQ ID NO:12.

47. The non-oligomerizing tandem fluorescent protein of claim 45, wherein the *Discosoma* RFP is a mutant of DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12, further comprising an I125R mutation.

48. The tandem non-oligomerizing fluorescent protein of claim 43, wherein the first fluorescent protein is a non-oligomerizing tandem fluorescent protein, and the second fluorescent protein is a non-oligomerizing fluorescent protein.

49. The tandem non-oligomerizing fluorescent protein of claim 48, wherein the non-oligomerizing fluorescent protein comprises a mutation of an amino acid residue corresponding to A206, L221, F223, or a combination thereof of SEQ ID NO:2.

50. The tandem non-oligomerizing fluorescent protein of claim 49, wherein the mutation corresponds to S65G/S72A/T203Y/H231L in SEQ ID NO:2.

51. The tandem non-oligomerizing fluorescent protein of claim 49, wherein the mutation corresponds to S65G/V68L/Q69K/S72A/T203Y/H231L in SEQ ID NO:2.

52. The tandem non-oligomerizing fluorescent protein of claim 49, wherein the mutation corresponds to K26R/F64L/S65T/Y66W/N146I/M153T/V163A/N164H/H231L in SEQ ID NO: 2.

53. The tandem non-oligomerizing fluorescent protein of claim 49, wherein the mutation corresponds to H148G in SEQ ID NO: 2.

54. A method for determining the pH of a sample, the method comprising:  
    contacting the sample with a first non-oligomerizing tandem fluorescent protein of claim 1, wherein the emission intensity of the first non-oligomerizing tandem fluorescent protein changes as pH varies between pH 5 and pH 10,  
    exciting the indicator; and  
    determining the intensity of light emitted by the first non-oligomerizing tandem fluorescent protein at a first wavelength, wherein the emission intensity of the first non-oligomerizing tandem fluorescent protein indicates the pH of the sample.

55. The method of claim 54, wherein the first non-oligomerizing tandem fluorescent protein comprises a *Discosoma* RFP or a fluorescent protein related to a *Discosoma* RFP.

56. The method of claim 55, wherein the *Discosoma* RFP is DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12 or a mutant of SEQ ID NO:12.

57. The method of claim 56, wherein the mutant of SEQ ID NO:12 comprises an I125R mutation.

58. The method of claim 54, wherein the sample is a biological tissue.

59. The method of claim 54, wherein the sample is a cell or a fraction thereof.

60. The method of claim 54, further comprising:

contacting the sample with a non-oligomerizing fluorescent protein,

wherein the non-oligomerizing fluorescent protein is different from the first non-oligomerizing tandem fluorescent protein,

wherein the emission intensity of the non-oligomerizing fluorescent protein changes as pH varies from 5 to 10, and

wherein the non-oligomerizing fluorescent protein emits at a second wavelength that is distinct from the first wavelength;

exciting the non-oligomerizing fluorescent protein;

determining the intensity of light emitted by the non-oligomerizing fluorescent protein at the second wavelength; and

comparing the fluorescence at the second wavelength to the fluorescence at the first wavelength.

61. The method of claim 60, wherein the non-oligomerizing fluorescent protein is a second non-oligomerizing tandem fluorescent protein.

62. The method of claim 54, wherein the first non-oligomerizing tandem fluorescent protein comprises a targeting sequence.

63. The method of claim 62, wherein the targeting sequence comprises a cell compartmentalization domain.

64. The method of claim 63, wherein the cell compartmentalization domain targets the first non-oligomerizing tandem fluorescent protein in a cell to cytosol, endoplasmic reticulum, mitochondrial matrix, chloroplast lumen, medial trans-Golgi cisternae, a lumen of a lysosome, or a lumen of an endosome.



65. The method of claim 64, wherein the cell compartmentalization domain comprises amino acid residues 1 to 81 of human type II membrane-anchored protein galactosyltransferase, or amino acids 1 to 12 of the presequence of subunit IV of cytochrome c oxidase.

66. A method for determining whether a sample contains an enzyme, the method comprising:

contacting a sample with a tandem non-oligomerizing fluorescent protein of claim 43;

exciting the donor, and

determining a fluorescence property in the sample,

wherein the presence of the enzyme in the sample results in a change in the degree of fluorescence resonance energy transfer.

67. A method for determining the activity of an enzyme in a cell, the method comprising:

providing a cell that expresses a tandem non-oligomerizing tandem fluorescent protein of claim 43,

wherein the peptide linker moiety comprises a cleavage recognition amino acid sequence specific for the enzyme coupling the donor and the acceptor,

exciting the donor, and

determining the degree of fluorescence resonance energy transfer in the cell,

wherein the presence of enzyme activity in the cell results in a change in the degree of fluorescence resonance energy transfer.

68. A method for identifying the presence of a molecule in a sample, the method comprising:

operatively linking a non-oligomerizing tandem fluorescent protein of claim 1 to the molecule, and

detecting fluorescence due to the non-oligomerizing tandem fluorescent protein in a sample suspected of containing the molecule, thereby identifying the presence of the molecule in the sample.

69. The method of claim 68, wherein the molecule is a polypeptide.

70. The method of claim 69, wherein the polypeptide is an antibody, an enzyme, or a receptor.

71. The method of claim 68, wherein the molecule is a polynucleotide.

72. The method of claim 68, wherein the sample is a biological sample.

73. The method of claim 72, wherein the biological sample comprises a cell, a tissue sample, or an extract of a cell or a tissue sample.

74. The method of claim 73, wherein said detecting is performed on an intact cell or tissue sample.

75. The method of claim 68, wherein said operatively linking comprises contacting the non-oligomerizing tandem fluorescent protein with the molecule under conditions suitable for linking the protein to the molecule.

76. The method of claim 68, wherein said operatively linking comprises expressing a recombinant nucleic acid molecule comprising a polynucleotide encoding the non-oligomerizing tandem fluorescent protein operatively linked to a polynucleotide encoding the molecule.

77. A method of identifying an agent or condition that regulates the activity of an expression control sequence, the method comprising:

exposing a recombinant nucleic acid molecule comprising a polynucleotide encoding a non-oligomerizing tandem fluorescent protein of claim 1 operatively linked to an expression control sequence to an agent or condition suspected of being able to regulate expression of a polynucleotide from the expression control sequence, and

detecting fluorescence of the non-oligomerizing tandem fluorescent protein due to said exposing, thereby identifying an agent or conditions that regulates expression of the expression control sequence.

78. The method of claim 77, wherein the expression control sequence is a transcription regulatory element.

79. The method of claim 78, wherein the transcription regulatory element is a promoter.

80. The method of claim 77, wherein the expression control sequence is a translation regulatory element.

81. The method of claim 77, wherein the condition comprises exposure to proteins expressed in a cell.

82. A method of identifying a specific interaction of a first molecule and a second molecule, the method comprising:

contacting the first molecule, which is operatively linked to a donor first non-oligomerizing tandem fluorescent protein, and the second molecule, which is operatively linked to an acceptor non-oligomerizing fluorescent protein, under conditions that allow a specific interaction of the first molecule and second molecule,

wherein the first non-oligomerizing tandem fluorescent protein and the non-oligomerizing fluorescent protein are different;

exciting the donor; and

detecting fluorescence resonance energy transfer from the donor to the acceptor, thereby identifying a specific interaction of the first molecule and the second molecule.

83. The method of claim 82, wherein the non-oligomerizing fluorescent protein is a second non-oligomerizing tandem fluorescent protein.

84. The method of claim 82, wherein the first molecule is a first cellular protein and the second molecule is a second cellular protein.

85. The method of claim 82, wherein first cellular protein and the second cellular protein are the same.

86. The method of claim 82, wherein the first molecule is a polynucleotide and the second molecule is a polypeptide.

87. The method of claim 86, wherein the polynucleotide is a transcription regulatory element and the polypeptide is a putative transcription factor.